AGRIFOOD METABOLOMICS - A LINK BETWEEN ANALYTICAL BIOCHEMISTRY AND GREEN BIOTECHNOLOGIES

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Abstract: We reviewed the most important lines of “omics’ developments, especially the terms and concepts which define metabolomics, with well documented, recent literature data. Recent data about metabolomics technologies (advanced analysis and chemometry) and their applications in applied biological sciences & technology are presented.

INTRODUCTION: TERMS AND CONCEPTS
The last two decades we assist to an exponential development of “omics”, biotechnology-related fields which aim the decoding of genomes (genomics - from bacteria to humans), the genetic expression in RNAs (transcriptomics) and proteins pool synthesized by translation (proteomics), useful for the synthesis of metabolites which confer the molecular profile, specific for a particular organism (metabolomics). Meanwhile, we assist to an expansion of “omics’” nomenclature, from agrogenomics, to “toxicomics” or “enzymomics’ and many others (9-11)

Biotechnology, the specific technology based on biology-related sciences, largely used in agriculture & food science, industry, environment and medicine, aims to obtain better performances of living organisms. Biotechnology use bioengineering and bioprocessing as tools to modify organisms, to improve their performances, by genetic engineering (fig 1.)

Fig.1. Metabolomics, in the context of Biotechnology development.
**Metabolomics** aims a systematic study of the chemical fingerprints of specific cellular processes – especially the small-molecule metabolite profiles (3,16,17,31). The **metabolome** represents the collection of all small metabolites in a biological organism (hormones, signalling molecules, secondary metabolites), which are the end products of its gene expression. Thus, while *genome* gives a general fingerprint of DNA content, *proteome* shows the mRNA gene expression, the metabolic profile (*metabolome*) gives a rapid “picture” of the cell physiology. One of the challenges of systems biology is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms (7, 12, 31).

Like the transcriptome and the proteome, the **metabolome** is dynamic, changing from second to second. Although the metabolome can be defined readily enough, it is not currently possible to analyse the entire range of metabolites by a single analytical method. The term **Metabonomics** is used as well and is defined as quantitative measurement of the dynamic, multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification (17, 18). The difference between the two terms is not related to choice of analytical platform: although metabonomics is more associated with NMR spectroscopy and metabolomics with mass spectrometry-based techniques, this is simply because of usages amongst different groups that have popularized the different terms. While 'metabolomics' places a greater emphasis on comprehensive metabolic profiling, regardless of species investigated, 'metabonomics' is used to describe multiple metabolic changes caused by a biological perturbation.

![Diagram of Agrifood-related OMICS and biomarkers to characterize phenotypes](image)

**Fig.2.** Agrifood-related OMICS and biomarkers to characterize phenotypes, included in systems’ biology.

**Metabolites** are small molecules, intermediates and products of metabolism. A primary metabolite is directly involved in the normal growth, development, and reproduction, while a secondary metabolite is not directly involved in main metabolic pathways, but has important ecological function (defence, reproduction), e.g. pigments, hormones, terpenoids, alkaloids,
antibiotics (phytoncides), phytoalexins. Metabolome is a generic term which include the large network of metabolic reactions, originated at Imperial College London and has been used in toxicology, disease diagnosis and a number of other fields ( )

METABOLOMIC TECHNOLOGIES

Metabolomics technologies are combined with those for genomics, transcriptomics and proteomics to provide the most complete picture of the fundamental molecular organisation of biological materials. We offer the most efficient, cost-effective platform for your needs (1, . Metabolic biochemists have been 'doing metabolomics' since 60's by chromatographic separation techniques, detection of metabolites via UV, Vis , PDA, MS, and recently NMR detection. The name metabolomics was used firstly in the 90s (20) and in 2004, the Metabolomics Society was formed to promote this field (http://metabolomicssociety.org ) (6, 21)

The field of plant and agrifood metabolomics exploded after 2000, due to the results and efforts of researchers Max Planck Institute for Plant Physiology, in Golm, Germany, Plant Research International from Wageningen University, Netherlands, as well other research units in USA, Sweden, UK. Their research on 'metabolite profiling' results in identifying hundreds of metabolites and not the entire complement of the plant cell with potential applications to agriculture, medicine, and other fields in the biological sciences (1, 13, 14, 15, 19, 23, 29, 30).

In 2007, the Human Metabolome Project, led by the University of Alberta, Canada (www.hmdb.ca), completed the first draft of the human metabolome, consisting of a database of approximately 2500 metabolites, 1200 drugs and 3500 food components. Similar projects have been underway in several plant species, most notably Medicago truncatula and Arabidopsis thaliana for several years.

Many of the bioanalytical methods used for metabolomics have been adapted or adopted from existing biochemical techniques and analytical chemistry-based analyses. Three characteristics common to metabolomic research are:

- it makes possible to profile metabolites with as little bias towards a specific metabolite or group of metabolites.
- it makes possible the profiling of a large number of metabolites at the same time, instead of being analyzed one by one.
- it makes possible, by multivariate methods to find significant relationships between the metabolites.

There are four important issues which are addressed for metabolite analysis: the efficient and unbiased extraction of metabolites from a biological tissue (1), the analytes separation usually by chromatography and capillary electrophoresis(2), the analytes detection (3) and their identification and quantification (4). The analytical methods applied in metabolomics are: Gas chromatography, especially when coupled with mass spectrometry (GC-MS), is one of the most widely used and powerful methods, with very high resolution, but requires generally chemical derivatization. Only volatile chemicals can be analyzed without derivatization while large, polar metabolites cannot be analysed by GC.

High performance liquid chromatography (HPLC) has lower chromatographic resolution than GC, but a much wider range of analytes which can be measured.

Capillary electrophoresis (CE) is most appropriate for charged analytes, it has a higher theoretical separation efficiency than HPLC, and is suitable for use with a wider range of metabolite classes than is GC, coupled or not with detection methods:

Mass spectrometry (MS) is used to identify and quantify metabolites after separation by GC, HPLC or CE. GC-MS is the most 'natural' combination and was the first to be developed. In
addition, mass spectral fingerprint libraries allow identification of a metabolite according to its fragmentation pattern. Recently it was developed the use MS as a stand-alone technology: the sample is infused directly into the mass spectrometer with no prior separation, and the MS serves to both separate and to detect metabolites.

Nuclear magnetic resonance (NMR) spectroscopy is the only detection technique which does not rely on separation of the analytes, and the sample can be recovered for further analyses. NMR is close to being a universal detector, although it is relatively insensitive compared to mass spectrometry-based techniques and difficult to interpret for complex mixtures.

MS and NMR are by far the two leading technologies for metabolomics. But other techniques, especially FTIR, NIR and Raman spectroscopy, electrochemical and isotopic detection are developing specifically for non-destructive analyses of metabolites, if preliminary validation is made against GC or LC methods.

All direct analytical data need sophisticated chemometry softwares and multivariant parameter analysis, in order to find significant relashionships between parameters (4, 28).

APPLICATIONS OF METABOLOMICS IN APPLIED BIOLOGICAL SCIENCES

Chemotaxonomy and Functional genomics applied in Agrifood. Metabolomics can be an excellent tool for determining the phenotype of plants, their genetic fingerprint (clusters based on their genetic origin) or caused by a genetic manipulation, detecting phenotypic changes in a genetically-modified plant intended for human or animal consumption. The prediction of unknown genes activity by comparison with the metabolic perturbations (experimental data coming from model organisms such as Saccharomyces cerevisiae and Arabidopsis thaliana).

Recently, the ‘phytochemical array’ concept refers to an integrated view of a given plant species characterisation according to its genome, transcriptome, proteome, metabolome and bioactivity of metabolites. The phytochemical array exhibits all of the links from the genome to the activity of metabolites for desired traits.

These tools have applications for crop improvement (e.g. molecular breeding of biotic and abiotic stress-resistant plants), to the discovery and development of plant-based pharmaceuticals, to the development of functional foods, and to the production of plant-derived industrial materials and energy. All of these advances will be valuable in the future and can be developed through genome-based plant biotechnology.

Our objectives and results obtained at the Research Center for Natural Products at USAMV Cluj-Napoca (www.biochim.usamvcluj.ro) are joining all aspects of plant and food metabolomics, using case studies of different plants cultivated in Romania (mainly medicinal plants) and their use in new food supplements or food products, as well investigations on food quality control, authenticity (for vegetable oils and fruit juices) and traceability. Complementary advanced analytical methods (GC-MS, LC-PDA, LC-MS and IR/NIR-based techniques) are applied for metabolic profiling.

Authenticity vs. adulteration, traceability and Food Quality Management. Based on specific metabolites’ fingerprint in the food chain, from raw materials (cereals, vegetables, etc.) to final food products, one can identify the biological (genetic) or geographical origin of products, the traceability of specific biomarkers, the authenticity of final food products, the impact of technology on the food quality, the possible adulterations by mixes of with low-quality food, identification of toxic ingredients or degraded bioactive molecules. All these aspects are covered in the new concept of Food Quality management which use advanced
technologies like Metabolomics to investigate and find solutions for a higher food quality offered to consumers (24).

Fig. 4. Chromatograms and spectrometric measurements used for fingerprinting plants and food from Romanian sources. A. Comparative GC-MS of different phytosterols in edible oils (5) B. HPLC-PDA fingerprint of phenolics in Echinacea sp. of different geographic origins (22). C. GC-FID fingerprint of fatty acids (24). D. FTIR fingerprint of Melissa sp. extracts of different origins (8). E. Principal Component Analysis as chemometry tool to analyse metabolome relations between different plants (26). F. UV-Vis spectrometry used to identify oils according to their specific pigment content (24)

**Nutrigenomics** links genomics, transcriptomics, proteomics and metabolomics to human nutrition. Generally a metabolome is influenced by genetic (age, sex, body composition, inherited pathology) and epi-genetic factors (environment, food quality, lifestyle and diet). Metabolomics determines a biological endpoint in nutrigenomics, the metabolic fingerprint, reflects the balance of all these forces on an individual's metabolism and nutrigenetics offers a way to repair, as profilaxy, the inherited organism sensibility or liability for certain nutrients (29).
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